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EXPERIMENTAL ARTICLES

New *Bacillus subtilis* Strains as Promising Probiotics

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Abstract—The properties of new *B. subtilis* strains GM2 and GM5, isolated from potato rhizosphere and possessing high antimicrobial activity, were studied. The potential of the strains for their use as probiotics was characterized. The strains were resistant to bile and to a wide range of the ambient pH. *B. subtilis* strains GM2 and GM5 possessed proteolytic and phytate-hydrolyzing activity and proved to be safe for model animals. The strains were characterized by antagonistic properties against phytopathogenic micromycetes, as well as against pathogenic and opportunistic enterobacteria. *B. subtilis* GM2 and GM5 were concluded to be promising strains for use as probiotics.

Keywords: Bacillus subtilis GM2 and GM5, characteristics of strains, proteolytic activity, antimicrobial activity, probiotics

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Probiotics are live microorganisms, which have a positive effect on diverse functions of an organism. In particular, they prevent the invasion of various pathogens. *Salmonella enterica* is one of the causal agents of human gastrointestinal disorders. The main sources of this pathogen are agricultural animals and poultry, which serve as reservoirs of *S. enterica*. One of the ways to combat the spread of pathogens is to develop various probiotics as an alternative to antibiotics (Huyghebaert et al., 2011; Li et al., 2016; Bai et al., 2017). Autochthonous lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* are usually used as probiotics; gram-positive spore-forming bacteria of the genus *Bacillus* are used as probiotics as well (Huyghebaert et al., 2011). Bacteria of the genus *Bacillus* are able to form endospores, which aids their survival under extreme conditions: high or low temperatures, radiation, nonoptimal pH, pressure, and the presence of toxic chemicals that damage the vegetative cells (Oh et al., 2017; Bernardeau et al., 2017). A dense multilayered spore shell helps bacilli to retain their activity while passing through the gastrointestinal tract (GIT). Some GIT regions form toxic environments for *Bacillus* due to anoxic conditions, low pH, and bile salts, as well as to extremely high concentration of commensal bacteria (up to 10^{12} cells per 1 g of content in the colon), which compete for nutrients and environment (Bernardeau et al., 2017). Experiments with mice proved that *B. subtilis* spores were able to germinate, and the cells could proliferate and form the spores again in intestines of animals (Hoa et al., 2001; Bernardeau et al., 2017). Being potential probiotics, *B. subtilis* strains are widely studied at genetic and physiological levels (Cutting et al., 2011).

Studies have revealed that in addition to the resistance of the probiotic *Bacillus* strains to bile acids, they were capable of immune stimulation in case of gastrointestinal disorders (Endres et al., 2011). Ability to synthesize antibiotics, bacteriocins, cyclic lipopeptides, and lytic enzymes with antimicrobial activity provides probiotic activity for bacteria of the genus *Bacillus* (Sorokulova, 2013). Being probiotics, the strains of *B. subtilis* and *B. coagulans* were shown to have a growth-stimulating and prophylactic effect on broiler chickens (Guo et al., 2017; Oh et al., 2017).

We isolated two strains of *B. subtilis* from the potato rhizosphere, which exhibited high antimicrobial activity (Mardanova et al., 2017). The goal of the present work was to characterize the properties of these new strains and to assess their potential use as probiotics.

MATERIALS AND METHODS

The subjects of the study were *B. subtilis* strains GM2 and GM5 with high antimicrobial activity (Mardanova et al., 2017). Opportunistic coliform bacteria, *Salmonella enterica* serotype Typhimurium ATCC14028s, *Klebsiella oxytoca*, and *Escherichia coli*, as well as micromycetes *Fusarium avenaceum*, *F. oxysporum*, *F. redolens*, and *F. solani*, were used as test cultures.